Open Access Library Journal
2018, Volume 5, e4455
ISSN Online: 2333-9721
ISSN Print: 2333-9705

The Efficacy of *Ocimum gratissimum* Leaf Powder and Ethanol Extract on Adult *Periplanata americana* under Laboratory Condition

Boate Rosemary Ukoroije, Jasper Freeborn Nestor Abowei, Richard Abalis Otayoor

Department of Biological Sciences, Faculty of Science, Niger Delta University, Wilberforce Island, Nigeria
Email: rukoroije@gmail.com, cebealse@yahoo.com, richardotami@yahoo.com

How to cite this paper: Ukoroije, B.R., Abowei, J.F.N. and Otayoor, R.A. (2018) The Efficacy of *Ocimum gratissimum* Leaf Powder and Ethanol Extract on Adult *Periplanata americana* under Laboratory Condition. *Open Access Library Journal*, **5**: e4455.
https://doi.org/10.4236/oalib.1104455

Received: February 28, 2018
Accepted: April 23, 2018
Published: April 26, 2018

Abstract

The efficacy of *Ocimum gratissimum* leaf powder and ethanol extract on adult *Periplanata americana* under laboratory condition was studied to control the nuisance of the pest. The mean mortality count of adult *Periplanata americana* was highest (84) in 50 g leaf powder and lowest (3) in the control. The highest mortality (125) was recorded in 96 hr exposure time while the lowest (17) was recorded in 24 h exposure time. There was significant difference (P-value = 0.05) in the mortality with increase in grams and exposure time of leaf powder and the control at 5% level of significance. The mean mortality count of adult *P. americana* was highest (104) in 50 ml ethanoic extract and lowest (1) in the control. The highest mortality (143) was recorded in 96 hr exposure time while the lowest (55) was recorded in 24 h exposure time. There is significant difference (P-value = 0.05) in the mortality with increase in grams and exposure time of leaf powder and the control at 5% level of significance. The efficacy of *O. gratissimum* Ethanol extract is more than the leaf powder. There was significant difference in mortality in both increase in concentration levels and exposure time at 5% level of significance in both leaf powder and ethanol extract.

Subject Areas

Agricultural Science

Keywords

Scent Leaf Powder/Ethanol Extract, Cockroach, Laboratory Condition

1. Introduction

Ocimum gratissimum (scent leaf) is made up of different subspecies varieties and formas. The division was based on differences in fruiting calyx, content and morphology. The existing chromosome counts of *O. gratissimum* are: 2n = 40, 48, 64). Forma caryophyllatum is characterized by: leaves clove-scented when bruised, upper side short-haired, lower side densely gland-dotted, bracts 4 - 6 mm long, much longer than wide, lower lip of corolla not flushed with violet; and forma graveolens by: leaves strongly odoriferous but not clove-scented when bruised, upper surface covered with minute hairs, bracts 2 - 4 mm long, about as long as wide, lower lip of corolla flushed violet inside. Many Ocimum species contain essential, primarily used as vegetable (*O. americanum*), spice (*O. basilicum*.), or vegetable and medicine (*O. tenuiflorum*) [1].

Scent leaf is a perennial plant with sweet aroma. Its height ranged from 1 - 3 m tall (**Plate 1**). The stem is erect with round-quadrangular, multi branched woody base and epidermis strips peeling. Leaves opposite; petiole 2 - 4.5 cm long, slender, pubescent; blade elliptical to ovate, 1.5 - 16 cm × 1 - 8.5 cm, membranaceous, sometimes glandular punctuate, base cineaste which are entire, coarsely crenate-serrate margin, acute pubescent apex, verticillaster inflorescence, in a terminal, simple arrangement with lax rachis, soft pubescent; sessile bracts, ovate ranging from 3 - 12 mm × 1 - 7 mm and acuminate, caducous; pedicel 1 - 4 mm long, spreading or ascending, slightly curved; flowers in 6-10-flowered verticillasters and small. It is and hermaphrodite with 2-lipped calyx, 2 - 3 mm long, in fruit ranging from 5 - 6 mm with pubescent, upper lip rounded fruit, lower lip with 4, narrow, pointed spines. The central pair of teeth minute are much shorter than the upper lip with corolla campanula raging from 3.5 - 5 mm long, 2-lipped, greenish-white, pubescent outside, upper lip truncate, 4-fid, lower lip longer, declinate, flat, entire; stamens 4, declinate, in 2 pairs, inserted on the corolla tube, filaments distinctly exserted, upper pair with a

Plate 1. *Ocimum gratissimum* (Scent leaf) plant, Source: 220px-starr_030202-005_*Ocimum-gratissum.*

bearded tooth at the base; ovary superior, consisting of 2 carpels, each 2-celled, style 2-fid. Fruit consisting of 4, dry, 1-seeded nutlets enclosed in the persistent calyx (the lower lip closing the mouth of the fruiting calyx); nutlet subglobose, 1.5 mm long, rugose, brown; outer pericarp not becoming mucilaginous in water [1].

Flowering started after 136 days and continued until 195 days. Seed matured after 259 days. Flowering and seed set were much poorer than in *O. basilicum* L. or *O. minimum* L. In South-East Asia flowers can be found year-round. In northern India, oil content of young plants was low (2.3%) until the seed setting stage, then remained constant at 2.8% until the seed maturation stage. In its native area *O. gratissimum* occurs from sea-level up to 1500 m altitude in coastal scrub, along lake shores, in savanna vegetation, in submontane forest, and disturbed land. In South-East Asia it is not frequently found in open locations like roadsides and clearings, but more often cultivated as a hedge plant, up to about 300 m altitude. Its biophysical limits Altitude: 0 - 1500 m [1].

O. gratissimum is grown for the essential oil in its leaves and stems. Eugenol and to a lesser extent thymol extracted from the oil are substitutes for clove oil and thyme oil. The essential oil is also an important insect repellent. The whole plant and the essential oil have many applications in traditional medicine, especially in Africa and India. Preparations from the whole plant are used as stomachic and in treating sunstroke, headache and influenza. The seeds have laxative properties and are prescribed against gonorrhoea. The essential oil is applied against fever, inflammations of the throat, ears or eyes, stomach pain, diarrhoea and skin diseases. It is being tested as an antibiotic. In Indonesia (Sumatra) a tea is made from the leaves, while in Thailand the leaves are applied as a flavouring. In Indonesia the eugenol-type of *O. gratissimum* is used in the ceremonial washing of corpses and is planted in graveyards. In India *O. gratissimum*, named "ram tulsi", is widely used in religious ceremonies and rituals [1].

The fresh aboveground parts of *O. gratissimum* contain 0.8% - 1.2% essential oil. The chemical composition of the oil is variable and at least 6 chemotypes have been reported, characterized by the main component of the essential oil: eugenol, thymol, citral, ethyl cinnamate, geraniol and linalool. An overview of the occurrence of the various types and possible implications for the taxonomy is lacking. The eugenol type is the most important economically; the thymol type was formerly important, but most thymol is now produced synthetically, while natural thymol is mostly obtained from *Thymus vulgaris* L. or *Trachyspermum ammi* (L.) Sprague ex Turrill. The other types are of little economic importance. The eugenol-type oil is a brownish-yellow to pale yellow liquid with a powerful, warm-spicy and aromatic odour, reminiscent of clove oil, but with a sweet-woody, almost floral top note. The dry-out is bitterer than that of clove oil. Analysis of a sample of an essential oil of the eugenol type from Vietnam indicated that the main component was eugenol (71%) with small amounts of D-germacrene and (Z)-beta-ocimene. In a sample from southern China the eu-

genol content was as much as 95%. Samples from Madagascar had eugenol contents of 40% - 90%, with very variable other components [1].

The thymol-type oil is a dark yellow to orange-yellow or brownish liquid with a medicinal-spicy, warm and somewhat herb-like odour. Its flavour is warm, slightly astringent and burning, and has a sweet medicinal aftertaste. Analysis of several samples of essential oils from *O. gratissimum* from Central and West Africa rich in thymol indicated that their main constituents were thymol, gamma-terpinene, p-cymene and eugenol. The concrete obtained by solvent extraction is much richer in thymol than the distilled oil. A geraniol-rich type, found in the United States, contained mainly geraniol (84% - 88%) with small amounts of gamma-muurolene, neral, beta-caryophyllene and limonene. The citral type, reported from Iran, Pakistan and India, is rich in citral (67%) and geraniol (26%). *O. gratissimum* is also cultivated as a hedge plant [1].

The time for transplanting seedlings into the field in the delta of the Hong River in northern Vietnam is February-March, in southern Vietnam from May-August. Plants are spaced at about 40 cm × 50 cm. The optimum harvesting time for distillation of the essential oil is when 3 branches per plant or 75% of the branches are flowering. In northern Vietnam 2 - 3 cuts can be obtained in an average year, 4 - 5 cuts per year in the south. In Vietnam, *O. gratissimum* remains productive for 5 - 10 years. In India, yields of 70 - 75 t/ha green herbage of *O. gratissimum* producing 400 L essential oil in 2 years have been obtained experimentally. In Thailand harvesting every 10 - 12 days resulted in an annual green herbage yield of only 13 t/ha and an oil yield of nearly 200 l.

The American cockroach (*Periplaneta americana*), also colloquially known as the water bug, but not a true water bug since it is not aquatic, or misidentified as the palmetto bug. It is the largest species of common cockroach, and often considered a pest. It is also known as the ship cockroach, kakerlac, and Bombay canary. Despite the name, none of the *Periplaneta* species is endemic to the Americas; *P. americana* was introduced to the United States from Africa as early as 1625. They are now common in tropical climates because human activity has extended the insect's range of habitation, and are virtually cosmopolitan in distribution as a result of global commerce. American cockroaches (**Plate 2**) are also known as plagues in the warm Meditation coast of Spain of, as well as in southern Spain and southern Portugal (starting from and in the canary Island; where the winters are mild/warm and frost-free, and the summers are hot. Cockroaches date back to the carboniferous period. They are thought to have emerged on the supercontinent Pangaea, or on Gondwana, the daughter continent of Pangaea. The cockroach made many adaptations over the years to be able to survive the major die-offs to which many species succumbed. However, like all the extant species, the American cockroach has probably evolved in the last few millions to thousands of years and is a fully modern organism.

American cockroach has an average length of around 4 cm (1.6 in) and about 7 mm (0.28 in) tall. They are reddish brown and have a yellowish margin on the body region behind the head. Immature cockroaches resemble adults except they

Plate 2. The American cockroach: *Periplaneta americana.*

are wingless. The cockroach is divided in three sections; the body is flattened and broadly oval, with a shield-like pronotum covering its head. A pronotum is a plate-like structure that covers all or part of the dorsal surface of the thorax of certain insects. They also have chewing mouth parts, long, segmented antennae, and leathery forewings with delicate hind wings. The third section of the cockroach is the abdomen.

The insect can travel quickly, often darting out of sight when someone enters a room, and can fit into small cracks and under doors despite its fairly large size. It is considered one of the fastest running insects.

In an experiment, *P. americana* registered a record speed of 5.4 km/h (3.4 mph), about 50 body lengths per second, which would be comparable to a human running at 330 km/h (210 mph). It has a pair of large compound eyes, each having over 2000 individual lenses, and is a very active night insect that shuns light. The American cockroach shows a characteristic insect morphology with its body bearing divisions as head, trunk, and abdomen. The trunk, or thorax, is divisible in prothorax, mesothorax and metathorax. Each thoracic segment gives rise to a pair of walking appendages (known as legs). The organism bears two wings. The forewings, known as tegmina arises from mesothorax and is dark and opaque. The hind wings arise from metathorax and are used in flight, though they rarely do. The abdomen is divisible into ten segments each of which is surrounded by chitinous exoskeleton plates called sclerites, including dorsal tergites, ventral sternites and lateral pleurites. The odorous secretions produced by American cockroaches can alter the flavor of food. Also, if populations of cockroaches are high, a strong concentration of this odorous secretion can be present. Cockroaches can pick up disease-causing bacteria, such as Salmonella on their legs and later deposit them on foods and cause food infections or poisoning. House dust containing cockroach feces and body parts can trigger allergic reactions and asthma in certain individuals.

American cockroaches generally live in moist areas, but can survive in dry

areas if they have access to water. They prefer warm temperatures around 29˚C (84˚F) and do not tolerate cold temperatures. These cockroaches are common in basements, crawl spaces, cracks and crevices of porches, foundations, and walkways adjacent to buildings. In residential areas outside the tropics, these cockroaches live in basements and sewers, and may move outdoors into yards during warm weather. American cockroaches have three developmental stages: egg, nymph and adult. Females produce an egg case (ootheca) which protrudes from the tip of the abdomen. On average, females produce 9 - 10 oothecae, although they can sometimes produce as many as 90. The cockroach is paurometabolous. After about two days, the egg cases are placed on a surface in a safe location. Egg cases are about 0.9 cm (0.35 in) long, brown, and purse-shaped. Immature cockroaches emerge from egg cases in 6 - 8 weeks and require 6 - 12 months to mature. After hatching, the nymphs feed and undergo a series of 13 moultings (ecdysis).

Adult cockroaches can live up to an additional year, during which females produce an average of 150 young. American cockroaches are omnivorous and opportunistic feeders that eat a great variety of materials such as cheese, beer, tea, leather, bakery products, starch in book bindings, manuscripts, glue, hair, flakes of dried skin, dead animals, plant materials, soiled clothing, and glossy paper with starch sizing. They are particularly fond of fermenting foods. They have also been observed to feed upon dead or wounded cockroaches of their own or other species. In cold climates, these cockroaches may move indoors when the weather turns cold, seeking warmer temperatures and food. Cockroaches may enter houses through sewer connections, under doors, or around plumbing, air ducts, or other openings in the foundation. Cockroach populations may be controlled through the use of insecticides. Covering any cracks or crevices through which cockroaches may enter and cleaning any spills or messes that have been made is beneficial, so cockroaches are not able to enter and are not attracted to the food source. Another way to prevent an infestation of cockroaches is to thoroughly check any material brought inside. Cockroaches and egg cases can be hidden inside or on furniture, in boxes, suitcases and grocery bags among others. **Table 1** is a comparison of three common cockroaches.

The threat of insects and other pests particularly cockroaches, is well known and have been a challenge to man. Cockroaches are the most abundant insect pest of public health importance; they infest hospitals, food manufacturing industries, kitchens and residential apartments [2]. Cockroach infestation has always raised safety concerns, especially as carriers of food-borne pathogens and food spoilage organisms [3]. As they feed on materials, cockroaches leave filth and secrete oily liquid having offensive and sickening odour that ruin food [4]. Cockroaches feed on human excreta as well as human food, thus are potential transmitters of diseases such as dysentery, typhoid, cholera and other food-borne infections which have been experimentally confirmed [5]. Dust containing cockroach excreta triggers allergen reaction such as wheezing in many individuals, making it particularly harmful to asthmatic patients. A robust association has

Table 1. Comparison of three common cockroaches.

Roach	German cockroach	Oriental cockroach	American cockroach
Size	13 - 16 mm (0.51 - 0.63 in)	18 - 29 mm (0.71 - 1.14 in)	29 - 53 mm (1.1 - 2.1 in)
Preferred temperature	15˚C - 35˚C (59 - 95˚F)	20 - 30˚C (68 - 86˚F)	20˚C - 29˚C (68 - 84˚F)
Nymphal development	54 - 215 days (at 24˚C - 35˚C (75 - 95˚F)	164 - 542 days (at 22˚C - 30˚C (72 - 86˚F))	150 - 360 days (at 25˚C - 30˚C (77 - 86˚F))
Lifespan	Around 200 days	35 - 190 days	90 - 706 days
Able to fly	Uncommon	No	Yes

Source: https://en.wikipedia.org/wiki/americn_cocroch#cite_note-Robinson2005-18.

been established between the presence of cockroaches and increase in the severity of asthma symptoms in individuals sensitive to cockroach allergen [6] [7]. Cockroaches are the most persistent pest to have colonized the planet. Since their origin, not only do they present a variety of health hazard when found in our houses, but are also a threat in commercial places, spreading diseases through any food source, that they come in contact with [6]. Cockroaches can cause food poisoning by crawling into food materials [7]. Cockroach bite in humans lead to an extremely infested wound, so cockroach infection should be treated immediately.

The presence and rising population of cockroach in our homes, especially the kitchen, poses great problems. In spite of so many pesticides used continuously, it seems as these pesticides although kill directly some of it, however cause side effects to humans [6]. Cockroach as pests, pose many health hazards to people. They also cause the destruction of tangible assets and can be damaging to human health too. They damage fabrics, book bindings and foods. This is largely due to the fact that they excrete only liquid which is not only dirty and filthy but carries an odour which is offensive to the nose, and can contaminate food substances [6]. Cockroach control is carried out using pesticides in form of chemical or gels, however, these alone are not 100% effective against the pest menace as the chemicals are directly or indirectly toxic, therefore an integrated pest management program is required. The ancient man had deployed different methods of control, including prayers, magic spells, cultivation systems, mechanical practices as well as application of organic and inorganic substances [8].

Between 500 BC and the 19th century a number of substances which were classified as pesticides and defined as "any substances or mixture of substances for preventing, destroying, repelling or mitigating any pest" were used to control pests. The best pest control method is that which is non-toxic and environment friendly, hence the use of natural plant parts as bio-pesticides. To overcome the problems of synthetic chemical hazards, the use of plant origin product is considered the best control measure which has become popular due to their degradability, least persistence and least toxicity to non-target organisms, economical and easy availability. About 200 plants with insecticidal activities are known

including *Azadirachta indica* (neem) tree whose insecticidal properties have proven successful in the control of over 550 insect species such as the orders Dictyoptera (cockroaches and mantis), Coleopera, Isoptera, Homoptera, Heteroptera, Diptera Orthoptera and others [9]. Developing countries and Nigeria in particular face the most challenges in achieving the sound management of pesticides. A large proportion of the population in Nigeria is directly engaged in agricultural work, often on a very small scale. While evidences abound that botanical pesticides are generally safe and effective [10], their use in Nigeria as in other parts of Africa is still hampered by some challenges such as most data on botanical pesticides been obtained from laboratory trials; field data are rare.

There is still hardly any developed appropriate technology for the application of botanicals, especially the oil and dust formulations [11]. Also compared with synthetic insecticides, the effects of natural insecticides are short-lived thus frequent applications are required to obtain reasonable degree of crop protection? Furthermore, bio pesticides formulations are yet to be available in usable forms to farmers in commercial quantities so as to serve as alternatives to synthetic pesticides. From the foregoing, the need to advocate for and implement integrated pest management strategies both on field pests, storage product pests, structural pests and domestic pests is indispensable. Therefore the discovery advocating for the adoption and promotion of the use of bio pesticides like *Ocimum gratissimum* in an integrated pest management frame work is quite relevant.

2. Materials and Methods

Fresh leaves of *Ocimum gratissimum* were collected form Amassoma Town in Southern Ijaw Local Government Area, Bayelsa State, Nigeria. They were identified at the herbarium of the University of Port Harcourt, Rivers State, Nigeria. The leaves of *O. gratissimum* were separated manually. The plant leaves were oven dried under the temperature of 60˚C overnight [12]. The dried leaves were ground into powder using electric blender (Binatone model) and sieved to obtain fine powders. The plant powders were put in air tight containers separately to ensure that the active ingredients are not lost. The powders were stored in a cool dry place until when needed.

For the extract, 1.5 kg of the powder was mixed with 2.8 liters of Ethanol and the mixture was allowed to stand overnight in the refrigerator. The following morning, this mixture was blended and filtered. The filtrate was evaporated using a desiccator and the resultant yield was 60 g of the extract which was about 4% yield. This was preserved in the refrigerator at 40c until ready for use. The total of Two hundred and forty (240) adult American cockroach used for the experiment were reared in a plastic container under ambient laboratory temperature of 30˚C ± 3˚C and 75% ± 3%. *P. americana* was fed with bread crumbs. One hundred *P. americana* were introduced into each treatment. All Treatments were arranged in completely randomized design (C.R.D).

Data Collection and Statistical Analysis Data were generated and recorded from mortality count of adult *P. americana* at 24, 48, 72 and 96 hrs were used to determine the most efficient proportions of the powders. Dead weevils were removed and discarded after every count. Data generated on mortality of the weevils due to efficacy of leaf powders were subjected to analysis of variance (ANOVA) using SPSS computer Software package (version 20) at 0.05 significant levels.

3. Results

The mean mortality of adult *Periplanata americana* exposed to *Ocimum gratissum* leaf powder is presented in Table 2. The result showed that the mean mortality count of adult *Periplanata americana* was highest (84) in 50 g leaf powder and lowest (3) in the control. The result also showed that the highest mortality (125) was recorded in 96 hr exposure time while the lowest (17) was recorded in 24 h exposure time. The Analysis of Variance (ANOVA) for the mean mortality in the experiment and the control indicated that there was significant difference (P-value = 0.05) in the mortality with increase in grams and exposure time of leaf powder and the control at 5% level of significance (Table 3).

The mean mortality of adult *P. americana* exposed to *O. gratissum* ethanoic extract is presented in Table 4. The result showed that the mean mortality count of adult *P. americana* was highest (104) in 50 ml ethanoic extract and lowest (1) in the control. The result also showed that the highest mortality (143) was recorded in 96 hr exposure time while the lowest (55) was recorded in 24 h exposure time. The Analysis of Variance (ANOVA) for the mean mortality in the experiment and the control indicates that there is significant difference (P-value = 0.05) in the mortality with increase in grams and exposure time of leaf powder and the control at 5% level of significance (Table 5). The result Table 2 and Table 4 show that the efficacy of *O. gratissimum* Ethanol extract is more than the

Table 2. Mean mortality of adult *Periplanata americana* exposed to *Ocimum gratissum* leaf powder.

Leaf Powder Quantity (g)	Exposure Time (Hrs)					
	24	48	72	96	Total	MN ± S.E
00	0	0	1	2	3	0.8 ± 0.5
10	0	3	10	17	30	7.5 ± 3.8
20	0	10	17	23	50	12.5 ± 4.9
30	2	14	19	26	61	15.3 ± 5.1
40	5	16	23	27	71	17.8 ± 4.8
50	10	19	25	30	84	21.0 ± 4.3
Total	17	62	95	125	295	-
MN ± S.E	2.83 ± 1.64	10.33 ± 3.06	15.83 ± 3.66	20.83 ± 4.17	-	-

Table 3. Analysis of mortality rate in adult *P. americana* exposed to *O. gratissum* leaf powder.

Descriptive statistics					ANOVA					
Groups	Sample size	Sum	Mean	Variance	Source of Variation	d.f.	SS	MS	F	p-value
24 Hr	6	17	2.83	16.17	Between Groups	3	1072.13	357.38	5.56	0.29
48 Hr	6	62	10.33	56.27	Within Groups	20	1285.83	64.29	-	-
72 Hr	6	95	15.83	80.17	Total	23	2357.96	-	-	-
96 Hr	6	125	20.83	104.57	Residual standard error	8.02	-	-	-	-
Total	24	-	12.46	102.52	Hartley Fmax (d.f. = 4, 5)	6.47	-	-	-	-
					Cochran C (d.f. = 4, 5)	0.41	-	-	-	-
					Bartlett Chi-square (d.f. = 3)	3.72	-	-	-	-

Table 4. Mean mortality of adult *Periplanata. americana* exposed to *Ocimum gratissum* ethanoic leaf extract (ml).

Ethanoic Leaf Extract (ml)	Exposure Time (Hrs)					
	24	48	72	96	Total	MN ± S.E
00	0	0	1	0	1	0.5 ± 0.3
10	3	10	13	23	49	11.0 ± 4.8
20	7	17	20	30	74	16.3 ± 4.7
30	11	20	23	30	84	18.8 ± 4.3
40	15	23	27	30	95	22.0 ± 3.7
50	19	25	30	30	104	24.3 ± 3.5
Total	55	95	114	143	407	-
MN ± S.E	6.89 ± 2.55	12.8 ± 3.55	20.0 ± 4.16	22.17 ± 4.43	-	-

Table 5. Analysis of mortality rate in adult *P. americana* exposed to *O. gratissum* ethanoic leaf extract (ml).

Descriptive statistics					ANOVA					
Groups	Sample size	Sum	Mean	Variance	Source of Variation	d.f.	SS	MS	F	p-value
24 *Hr*	6	41.	6.83	38.97	Between Groups	3	881.46	293.82	3.49	0.03
48 *Hr*	6	77.	12.83	75.77	Within Groups	20	1682.5	84.13	-	-
72 *Hr*	6	120.	20.	104.	Total	23	2563.96	-	-	-
96 *Hr*	6	133.	22.17	117.77	Residual standard error	9.17	-	-	-	-
Total	24	-	15.46	111.48	Hartley Fmax (d.f. = 4, 5)	3.02	-	-	-	-
					Cochran C (d.f. = 4, 5)	0.35	-	-	-	-
					Bartlett Chi-square (d.f. = 3)	1.5	-	-	-	-

leaf powder. There was significant difference in mortality in both increase in concentration levels and exposure time at 5% level of significance in both leaf powder and ethanol extract.

4. Discussion

Biopesticides or plant based fumigants have long been reported as attractive alternatives to synthetic fumigants for the management of arthropods because botanicals pose little threat to the environment or to human health [13]. The result of this study showed that *Ocimum gratissum* the leaf powders and ethanoic extracts effective biocides for cockroach control. It also showed that the efficacy of *O. gratissimum* Ethanol extract is more than the leaf powder. Orwa *et al.* [1] reported that *O. gratissimum* is grown for the essential oil in its leaves and stems. Eugenol and to a lesser extent thymol extracted from the oil are substitutes for clove oil and thyme oil. The essential oil is also an important insect repellent. *Ocimum* is known for its pesticide properties due to diverse group of compounds in its essential oil [14]. *O. gratissimum* essential oil is reported to contain bioactive constituents that are insecticidal and repellent [15].

The toxicity is due to the toxic effects of the compounds eugenol, mono and sesquiterpenoids found in the plant extract. Sosan *et al.,* [16], studied on larvicidal activity of *O. gratissimum* on larvae of *Aedes aegypti* L. and found that oil exhibited 100% mortality at 300 mg/L concentration of 24 hours of exposure. Leaves and extracts of leaves of Ocimum species are reportedly useful as grain protectants in Eastern Africa. Jinq *et al*., [17] reported the use of leaves of Ocimum in controlling the life stages of mosquito. They concluded in their studies that Ocimum species have larvicidal action on mosquitoes. *O. gratissimum* has been used extensively in the traditional system of medicine in many countries. In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhea [18]. In the savannah areas, decoctions of the leaves are for mental illness [19]. The leaf *O. gratissimum* is used by the Ibos of the south eastern Nigeria in the management of the baby's cord, to keep the wound surfaces sterile [20]. It is also used in the treatment of fungal infections, cold and catarrh [21]. Brazilian tropical forest inhabitants used decoction of *O. gratissimum* roots as sedative for children. People of Kenya and sub-Saharan Africa used it in the treatment of abdominal pains, sore eyes, infections, coughs, barrenness, fever, convulsions and tooth gargle, regulation of menstruation and as a cure for the prolapsed of the rectum [22]. In India, the whole plant has been used for the treatment of sunstrokes, headache and influenza. The tribes of Nigeria use the leaves extract for the treatment of diarrhea, while the cold leaves infusions are used for the relief of stomach upset and haemorrhoids [23].

The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infection such as pneumonia, cough and other diseases such as diarrhea, headache, diseases of the eye, skin diseases, fever and conjunctivitis [24] [25]. Formulations of the leaf essential oil of *O. gratissimum* have

been incorporated in a variety of bases as topical antiseptics used in the treatment of minor wounds, boils, and pimples [26] [27]. The essential oil of *O. gratissimum* is said to contain eugenol and shows some evidence of antibacterial activity [28]. A test on guinea pigs found evidence that the essential oil relaxes the muscles of the small intestine, consistent with the traditional use of the plant to treat gastrointestinal disorder [29]. *O. gratissimum* is also said to have antitumor, anticancer potentials [30]. A study on rats also found evidence that the leaf extract of the plant prevented diarrhea [31]. *O. gratissimum* ethanolic extracts showed a hepaprotective effect [32] [33]. O*cimum* oil is active against several species of bacteria (*Escherichia coli*, shigella, salmonella and proteus), and fungi (*Trichophyton rubrum* and *Trichophyton mentagrophytes*) [34]. A previous screening of the crude extracts of plants used in traditional medicine showed that the essential oil of *O. gratissimum* inhibited growth of *Herpetomonas samuelpessoai* [35].

Antioxidants are substances that inhibit the oxidation of cells from toxins such as free radicals. The toxins can be from the natural digestion and metabolism of foods, alcohol, nicotine from cigarette smoke, environmental factors, OTC drugs (such as acetaminophen, (Tylenol) prescription drugs, and preservatives. Reactive oxygen species (ROS) including singlet oxygen ($1O_2$), superoxide ion (O_2^-), hydroxyl ion (OH^-), and hydrogen peroxide (H_2O_2) are highly reactive and toxic molecules generated in cells during normal metabolism. However, in response to a variety of factors including tobacco smoke, pollutants, ionizing radiations, alcohol, synthetic pesticides, and solvent, their production increases. ROS can cause oxidative damage to proteins, lipids, enzymes, and DNA, and they have also been linked to pathogenesis of oxidative diseases. Living cells possess an excellent scavenging mechanism to avoid excess ROS induced cellular injury, however, with ageing and under influence of external stresses, these mechanisms become inefficient, and dietary supplementation of synthetic antioxidants is required [36].

Aromatic plants such as *O. gratissimum*, particularly their essential oils, are being evaluated for antioxidant activity. *O. gratissimum* plant contains large amounts of antioxidants other than vitamin C, vitamin E, flavonoids and carotenoids. The presence of many pharmacologically active compounds in *Ocimum* species provides them protection against free radical induced oxidative damage of cellular components. The antioxidant capacity of basil essential oils has been studied several times [37]. The comparative antioxidant activity of essential oils of four *Ocimum* species (*O. basilicum, O. canum, O. gratissimum* and *O. sanctum*) by DPPH bioassay and found that *O. gratissimum* was the most anti-oxidative [38].

Oral and intraperitoneal acute toxicity and the subchronic intraperitoneal toxicity of the essential oil of *O. gratissimum* were investigated. The acute toxicity test involved oral and intraperitoneal administration of graded doses of Ocimum oil prepared as a 4% v/v emulsion to two groups each of 30 rats and mice.

LD50 and LD100 were determined for both routes and species. In the sub chronic toxicity study, 25 male Sprague-Dawley rats were randomized into four test groups and a control. Organs and blood samples were taken for analyses after a 30 days treatment period. A dose dependent sedative effect of Ocimum oil was observed during the acute toxicity study in mice and rats and in the sub-chronic test in mice and rats. Evidence of treatment, route, and dose-dependent toxicity were detected in both studies [39]. Change in weight of the testes, hearts, kidney, intestines and lungs of the rats were statistically insignificant. Data analysis of blood biochemical, haemotological and histopathological findings showed significant differences between control and treated groups and revealed that *O. gratissimum* essential oil is capable of invoking an inflammatory response that transits from acute to chronic on persistent administration [40]. While the study revealed that Ocimum oil might be better tolerated orally for systemic delivery, the oil has toxic potentialities that should not be overlooked [41]. It was also reported that *O. gratissimum* can affect macrophage functioning and can also be hepatocarcinogenic [42].

Essential oils derived from several Ocimum species have been reported to be active against several Gram-positive and Gram-negative bacteria as well as against yeasts and fungi due to their terpenic constituents [43]. Recently, essential oils and extracts of certain plants have been shown to have antimicrobial effects [44]. Janssen *et al*., [45] investigated the antimicrobial potential of four Ocimum species viz., *O. canum, O. gratissimum*, *O. trichodon* and *O. urticifolium* grown in Rwanda against *E. coli*, *Bacillus subtilis*. As an example of anti-mycotic activity, the oil of *O. gratissimum* leaves was fungicidal at 78 mg/L for Microsporum gypseum while a concentration of 312 mg/L was required to inhibit growth of *Candida albicans* and *Cryptococcus neoformis* [46].

When mixed separately with stored cowpea seeds as pest control agents caused the mortality of adult CI. *maculatus* compared to the control (4.00 ± 1.41; 2.67 ± 1.41; 1.89 ± 1.17). This result agrees with previous studies. Raguraman and Singh [47]; Musa *et al.* [48]; Moses and Dorathy, [49] carried out on *A. indica* and *V. amygdalina* plants species as a potential botanical pesticides. Raguraman and Singh (2000) reported that neem tree has long been recognized for its pesticidal properties against insect pests. Moses and Dorathy [49] reported that bitter leaf gave the best protection against cowpea weevil when compared with garlic and ginger. The significant difference ($P < 0.05$) obtained in the mortality of C. *maculatus* by comparing the efficacy of *A. indica* leaf powder with the control is an indication that *A. indica* leaf powder has some insecticide properties capable of controlling pests of stored cowpea. This study also supports Girish and Shankarah, [50] who reported that neem tops the list of 2400 plant species that are reported to have pesticide properties and is regarded as the most reliable source of eco-friendly biopesticide properties. However, no significant difference exists between *V. amygdalina* and *A. indica* leaf powder and between *V. amygdalina* leaf powder and the control. In contrast to the findings of Brisibe *et al*., [51] who reported that, of the three botanical pesticides (*A. annua*; *A. indica* and

O. gratissimum) tested, the highest adult insect mortality rate was recorded in the treatment with the highest concentration (20 g/250g cowpea seeds) of dried and pulverized leaves respectively. They reported that at the concentration of 5 g of the tested botanicals, no significant difference existed among them but in the present study, the treatment with the highest concentration of 5 g of the leaf powders of *A. indica* on 30 g of cowpea seeds caused a significant difference in the mortality of *C. maculatus*. The implication of this study is that the leaf powders of the two plant species can serve as botanical pesticides with *A. indica* leaf powder showing more efficacy than the *V. amygdalina* leaf powder.

5. Conclusion

Ocimum gratissimum leaf powder and ethanol extract are very effective in the control of *Periplanata americana* and other insect pest. The ethanol extract is however more effective than the leaf powder and should be recommended for controlling the menace of American coachroach since it is void of adverse environmental pollution. To solve the menace of cockroaches, effort should be geared towards applying *O. gratissimum* leaf powder or its ethanoic extract to safeguard the adverse effects of conventional pesticides. Furthermore, *O. gratissimum* is cheap and readily available. However, further research is required to determine the efficacy of the scent leaf using higher concentrations and on a wide range of other common insect pests.

References

[1] Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Anthony, S. (2009) Agroforestree Database: A Tree Reference and Selection Guide Version 4.0. http://www.worldagroforestory.org/site/treedatabases.asp

[2] Kutrup, B. (2003) Cockroach Infestation in Some Hospitals in Trabzon. *Turkish Journal of Zoology*, **27**, 73-77.

[3] Bouamama, L., Sorlozano, A., Laglauia, A., Lebbadi, M., Aarab, A. and Gutiérrez-Fernández, J. (2007) Antibiotic Resistance Patterns of Bacterial Strains Isolated from *Periplaneta americana* and *Musca domesticus* in Tangler, Morocco. *The Journal of Infection in Developing Countries*, **4**, 194-201.

[4] Ghosh J. and Gayen, A. (2006) The Protozoan Fauna Living in the Digestive System of *Periplaneta americana* in Kolkata, West. Bengal, India. *Journal of Parasitic Diseases*, **7**, 455-459.

[5] Tatfeng, Y.M., Usuanlele, M.U. and Orukpe, A. (2005) Mechanical Transmission of Pathogenic Organisms: The Role of Cockroaches. *Journal of Vector Borne Diseases*, **4**, 129-134.

[6] Rauh, V.A., Ginger, B.C. and Robin, S.G. (2002) Deteriorated Honey Contributes to High Cockroach Allergen Levels in Inner-City Households. *Environmental Health Perspectives*, **110**, 323-327. https://doi.org/10.1289/ehp.02110s2323

[7] Sarinho, E., Schor, D., Veloso, M.A. and Rizzo, J.A. (2004) There Are More Asthmatics Homes with High Cockroach Infestation. *Brazilian Journal of Medical and Biological research*, **37**, 503-510. https://doi.org/10.1590/S0100-879X2004000400007

[8] Jitendra, K., Nitin, K. and Kulkarni, D.K. (2009) Plant-Based Pesticides for Control

of *Helicoverpa amigera* on Cucumis. *Asian Agricultural History*, **13**, 327-332.

[9] Mondal, T. and Mondal, D. (2012) A Review on Efficacy of *A. indica*. A Juss Based Biopesticides: An Indian Perspective. *Research Journal of Recent Sciences*, **35**, 445-516.

[10] Okrikata, E. and Auaso, C.E. (2008) Bio Efficacy of Various Neem Dust Formulation for the Control of *Sorghum stem* Borers 11: Effect on Stalk and Peduncle in the Semi-Arid Zones of Nigeria. *Yobe Journal of Environmental Development,* **1**, 57-74.

[11] Lale, N.E.S. and Mustupha, A. (2000) Potential of Combining Neem (*Azadirachta indica* A. Juss) Seed Oil with Varietal Resistance for the Management of Cowpea Bruchid, *Callosobruchus maculatus* (F) (Coleoptera: bruchidae). *Journal of Stored Products Research*, **36**, 215-222. https://doi.org/10.1016/S0022-474X(99)00035-1

[12] Aliero, A.A. and Abdullahi, L. (2009) Effect of Drying on the Nutrient Composition of *Vernonia amygdalina* Leaves. *Journal of Phytology*, **1**, 28-32.

[13] Dubey, N.K., Tiwari, T.N., Mandin, D., Andriamboavonjy, H. and Chaumont, J.P. (2000) Antifungal Properties of *Ocimum gratissimum* Essential Oil (Ethyl Cinnamate Chemotype). *Fitoterapia*, **7**, 567-569. https://doi.org/10.1016/S0367-326X(00)00206-9

[14] Deshpande, R.S. and Tipnis, H.P. (1997) Insecticidal Activity of *Ocimum basilicum*. *Pesticides*, **11**, 1-12.

[15] Keita, S.M., Vincent, C., Schmit, J.P., Ramaswamy, S. and Belanger, A. (2000) Effect of Various Essential Oils on *Callosobruchus maculatus. Journal of Stored Products Research*, **36**, 355-364. https://doi.org/10.1016/S0022-474X(99)00055-7

[16] Sosan, M.B., Adewoyin, F.B. and Adewunmi, C.O. (2001) Larvicidal Properties of 3 Indigenous Plant Oils on the Mosquito *Aedes aegypti*. *Nig Journal of Natural Products and Medicine*, **5**, 30-33. https://doi.org/10.4314/njnpm.v5i1.11719

[17] Jing, Y.D., Kim, M.K., Ahn, V.J. and Lee, H.S. (2002) Larvicidal Activity of Brazilian Plants against *Aedes aegypti* and *Culex pipiens* Pallers (Diptera: Culcilidae). *Agricultural Chemistry and Biotechnology*, **45**, 131-134.

[18] Effraim, K.D., Jacks, T.W. and Sodipo, O.A. (2003) Histopathological Studies on the Toxicity of *Ocimum gratissimum* Leaves Extract on some Organs of Rabbit. *African Journal of Biomedical Research*, **6**, 21-25.

[19] Akinmoladun, A.C., Ibukun, E.O., Afor, E., Obuotor, E.M. and Farombi, E.O. (2007) Phytochemical Constituent and Antioxidant Activity of Extract from the Leaves of *Ocimum gratissimum*. *Science Research Essay*, **2**, 63-166.

[20] Nweze, E.L. and Eze, E.E. (2009) Justification for the Use of *Ocimum gratissimum* in Herbal Medicine and Its Interaction with Disc Antibiotics BMC. *Complementary and Alternative Medicine*, **9**, 1-6. https://doi.org/10.1186/1472-6882-9-37

[21] Ijeh, I.I., Omodamiro, O.D. and Nwanna, I.J. (2005) Antimicrobial Effects of Aqueous and Ethanolic Fractions of Two Spices, *Ocimum gratissimum* and *Xylopia aethiopica*. *African Journal of Biotechnology*, **4**, 953-956.

[22] Matasyoh, L.G., Josphat, C.M., Francis, N.W., Miriam, G.K., Anne, W.T.M. and Titus, K.M. (2007) Chemical Composition and Antimicrobial Activity of the Essential Oil of *Ocimum gratissimum* L. Growing in Eastern Kenya. *African Journal of Biotechnology*, **6**, 760-765.

[23] Ijeh, I.I., Njoku, O.U. and Ekenza, E.C. (2004) Medicinal Evaluation of *Xylopia aethiopica* and *Ocimum gratissimum. Journal of Medical Aromatic Science*, **2**, 45-46.

[24] Adebolu, T.T. and Salau, A.O. (2005) Antimicrobial Activity of Leaf Extracts of *Ocimum gratissimum*on Selected Diarrhoea Causing Bacteria in South Western

Nigeria. *African Journal of Biotechnology*, **4**, 682-684. https://doi.org/10.5897/AJB2005.000-3126

[25] Saula, J.R. (2005) Antifungal Activities of *Ocimum gratisimum* towards Dermatophytes. *Journal of Ethnopharmacology*, **48**, 72-86.

[26] Orafidiya, L.O., Fakoya, F.A., Agbani, E.O. and Iwalewa, E.O. (2005) Vascular Permeability Increasing Effect of the Leaf Essential Oil of *Ocimum gratissimum* (Linn.) as a Mechanism for Its Wound Healing Property. *African Journal of Tradition*, **2**, 253-258.

[27] Nakamura, C.V., Ishid, K., Faccin, L.C., Filho, B.P., Cortez, D.A., Rozental, S., Souza, W. and Ueda, N. (2004) *In Vitro* Activities of Essential Oils from *Ocimum gratissimum* against Four Candida Species. *Research in Microbiology*, **155**, 579-586. https://doi.org/10.1016/j.resmic.2004.04.004

[28] Ilori, M., Sheteolu, A.O., Omonibgelin, E.A. and Adeneye, A.A. (1996) Antibacterial Activity of *Ocimum gratissimum* (Lamiaceae). 283-284.

[29] Socorro, V.F.M., Francisco, J.A., Matos, J.H., Leal, C. and David, N.C. (2002) Resultant Effects of the Essential Oil of *Ocimum gratissimum* on Isolated Ileum of the Guinea Pig. *Journal of Ethnopharmacology*, **81**, 1-4. https://doi.org/10.1016/S0378-8741(02)00049-1

[30] Ekunwe, S.I., Thomas, M.S., Luo, X., Wang, H., Chen, Y., Zhang, X. and Begonia, G.B. (2010) Potential Cancer-Fighting *Ocimum gratissimum* (OG) Leaf Extracts: Increased Anti-Proliferation Activity of Partially Purified Fractions and Their Spectral Fingerprints. *Ethnicity and Diseases*, **2**, 1-12.

[31] Rabelo, M., Souza, E.P. and Soares, P.M.G. (2005) Antinociceptive Properties of the Essential Oil of *Ocimum gratissimum* L. in Mice. *Brazil Journal of Medical Biology Research*, **36**, 521-524. https://doi.org/10.1590/S0100-879X2003000400016

[32] Surana, V. and Jain, D. (2010) Protective Effect of *Ocimum gratissimum* against Carbon Tetrachloride Induced Hepatic Damage in Rats. *Pharmacology Online*, **2**, 1111-1119.

[33] Arhoghro, E.M., Ekpo, K.E. and Ibeh, G.O. (2009) Effect of Aqueous Extract of Scent Leaf (*Ocimum gratissimum*) on Carbon Tetrachloride (CCl4) Induced Liver Damage in Albino Wister Rats. *African Journal of Pharmacy and Pharmacology*, **3**, 562-567.

[34] Oboh, O.J., Honeybell, I.M. and Enabuele, S.A. (2009) Nutritional and Antimicrobial Properties of *Ocimum gratissimum* Leaves. *Journal of Biological Science*, **9**, 377-380. https://doi.org/10.3923/jbs.2009.377.380

[35] Holetz, F.B., Ueda-Nakamura, T., Filho, B.P.D., Cortez, D.A.G., Morgado-Diaz, J.A. and Nakamura, C.V. (2003) Effect of Essential Oil of *Ocimum gratissimum* on the Trypanosomatid *Herpetomonas samuelpessoai*. *Acta Protozoologica*, **42**, 269-276.

[36] Halliwell, B. (1997) Antioxidants and Human Disease: A General Introduction. *Nutrition Review*, **55**, 44-52. https://doi.org/10.1111/j.1753-4887.1997.tb06100.x

[37] Julisni, H.R. and Simon, J.E. (2002) Antioxidant Activity of Basil. In: Janick, J., Whipkey, A., Eds., *Trends in New Crops and New Uses*, ASHS Press, Alexandrian, 575-579.

[38] Bunrathep, S., Palanuvez, C. and Ruangrungsi, N. (2007) Chemical Composition and Antioxidative Activities of Essential Oils from Four *Ocimum* Species Endemic to Thailand. *Journal of Health Research*, **21**, 201-206.

[39] Ayinla, M.T., Dada, S.O., Shittu, S.T., Olayaki, L.A., Akiode, A.O. and Ojulari, S.L. (2011) Anti-Hyperlipidemic Effect of Aqueous Leaf Extract of *Ocimum gratissi-*

mum in Alloxan Induced Diabetic Rats. *International Journal of Medical Science*, **3**, 360-363.

[40] Shittu, S.T., Oyeyemi, W.A., Lasisi, T.J., Shittu, S.A., Lawal, T.T. and Olujobi, S.T. (2016) Aqueous Leaf Extract of *Ocimum gratissimum* Improves Haematological Parameters in Alloxan Induced Diabetic Rats via Its Antioxidant Properties. *International Journal of Applied and Basic Medical Research*, **6**, 96-100. https://doi.org/10.4103/2229-516X.179016

[41] Ilondu, E.M., Egwunyenga, O.A. and Iloh, A.C. (2004) The Effects of Wild Basil (*Ocimum suave*) Labiateae: (Lamiaceae) Leaf Dust as Protectant of Cereals, against *Rhyzopertha dominica* (F) Coleoptera: (Bostrydae). *Journal of National Academy Advanced Science*, **3**, 129-134.

[42] Olonisakan, A., Oladimeji, M.O. and Lajide, L. (2006) Bioactivity of Steam Distilled Oils against the Cowpea Bruchids, *Callosobruchus maculatus* (F) Infesting Stored Cowpea Seeds. *Pakistan Journal of Biological Science*, **9**, 1271-1275. https://doi.org/10.3923/pjbs.2006.1271.1275

[43] Ladipo, M.K., Doherty, V.F. and Kanife, U.C. (2010) Phytochemical Screening and Antibacterial Investigation of the Extract of *Ocimum gratissimum* (Scent Leaf) on Selected Enterobacteriaceae, Production Agriculture and Technology. *African Journal of Agriculture*, **6**, 75-76.

[44] Lang, L. and Buchbauer, G. (2010) A Review on Recent Research Results (2008-2010) on Essential Oils as Antimicrobials and Antifungals. *Flavour Fragrance Journal*, **27**, 13-39. https://doi.org/10.1002/ffj.2082

[45] Janssen, A.M., Scheffer, J.J., Ntezurubanza, L. and Svendesen, A.B. (1998) Antimicrobial Activities of Some *Ocimum* Species Grown in Rwanda. *Journal of Ethnopharmacology*, **26**, 57-63. https://doi.org/10.1016/0378-8741(89)90113-X

[46] Mann, A. (2012) Phytochemical Constituents and Anti-Microbial and Grain Protectant Activities of Clove Basil (*Ocimum gratissimum*) Grown in Nigeria. *International Journal of Plant Research*, **2**, 51-58. https://doi.org/10.5923/j.plant.20120201.08

[47] Raguraman, S. and Singh, R.P. (2000) Biological Effects of Neem (*Azadirachta indica*) Seed Oil on an Egg Parasitoid, *Trichologramma chilonia*. *Journal of Economic Entomology*, **92**, 1274-1280. https://doi.org/10.1093/jee/92.6.1274

[48] Musa, A.K., Oyerinde, A.A. and Owolabi, F.O. (2009) Evaluation of the Efficacy of Mixed Leaf Powders of *Vernonia amygdalina* L. and *Ocimum gratissimum* Del. against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Academic Journal of Entomology*, **2**, 85-87.

[49] Moses, O. and Dorathy, O. (2011) Pesticidal Effect of Some Plant Materials for the Control of Weevils (*Callosobruchus maculatus*) in Some Varierties of Cowpea during Storage in Makurdi, Southern Guinea Agro-Ecological Zone of Nigeria. Entomological Society of Nigeria. 42nd Annual Conference Ibadan Book of Abstracts, 20 p.

[50] Girish, K. and Shankara, B.S. (2008) Neem—A Green Treasure. *Electronic Journal of Biology*, **4**, 102-111.

[51] Brisibe, E.A., Adugbo, S.E., Ekanem, U., Brisibe, F. and Figueira, G.M. (2011) Controlling Bruchid Pests of Stored Cowpea Seeds with Dried Leaves of *Artemisia annua* and Two Other Common Botanicals. *African Journal of Biotechnology*, **10**, 9586-9592. https://doi.org/10.5897/AJB10.2336